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AN AUTOMATED SYSTEM FOR USE IN COLLECTING VOLATILE CHEMICALS RELEASED FROM PLANTS

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Abstract—A system is described for the collection of volatiles produced by plants that minimizes stress on the plant in an environment that is free from chemical impurities. Air entering a volatile collection chamber containing a plant is purified using a nonwoven fabric media infused with charcoal. A multitasking, computer-automated system is described that can simultaneously collect volatilized chemicals from plants as well as monitor and record environmental conditions associated with those collections. Collection of up to 16 samples can be made in varying sampling order, flow rates, and user-specified time periods, without disturbing the sampling environment. During the same time period, this system is capable of simultaneously monitoring up to eight environmental parameters using any type of sensor with electrical signal outputs. A multiport base assembly was designed to fit around the base of the plant permitting air samples to be collected at the bottom of the chamber. The chamber can pass ambient light so the plant may follow its natural photocycles. The entire system can be configured for continuous laboratory duty or portable field use by utilizing components that run on DC voltages. For the purpose of testing the system's performance, we determined the periodicity of the release of volatiles from red and yellow flowering four o'clock plants, *Mirabilis jalaba* (Nyctaginaceae). The major chemical released from four o'clocks was identified as ocimene. The onset of release occurred between 1400 and 1600 hr and increased with time with maximum amount of ocimene released during 1800–2000 hr, followed by a decrease in emission. No ocimene was detected after 2400 hr. Determination of the amount of ocimene released per flower was calculated for the 1800- to 2000-hr time period. Based on the number of open flowers during the 1800- to 2000-hr period, yellow four o'clock's released 80.9 (±7.3 SD) ng/hr/flower, while the red flowers released 51.9 (±7.0 SD) ng/hr/flower.

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Key Words—Automated volatile collection system, plant volatiles, whole plant sampling, four o'clock plants, *Mirabilis jalaba*, volatiles collection.

INTRODUCTION

Many plants, ranging from crop species to ornamentals, emit volatile compounds in varying quantities for a variety of reasons. Chemicals released range from gases such as ethylene, ethane, and oxygen, as a result of biochemical processes within the plant, to complex terpenoids that are used to lure insects for pollination (Wyatt, 1983). Chemicals released by flowering plants have been investigated, and the circadian and diurnal rhythms of floral fragrance emissions of plants have been documented by several researchers (see for example, Matile and Altenburger, 1988; Altenburger and Matile, 1988, 1990; Loughrin et al., 1990a,b, 1991). Recently we identified the chemicals and periodicity of release from flowers of the night-blooming jessamine, *Cestrum nocturnum* L., that are attractive to female cabbage looper moths, *Trichoplusia ni* (Hübner) (Heath et al., 1992). The release of these chemicals was shown to correspond to periods of feeding activity of female cabbage loopers. The chemicals released were collected and identified as benzaldehyde, benzyl acetate, and phenylacetaldehyde. Flight-tunnel bioassays of female cabbage loopers demonstrated that the moths exhibited upwind-oriented flight and contact with dispensers after releasing an artificial blend of these compounds.

In addition to developing better chemical lures for monitoring insects, there is interest in the volatiles released from plants in response to environmental changes or plant stresses such as lack of nutrients and water and stress due to disease (Kimmerer and Kozlowski, 1982; Biddington, 1986). Recent research has demonstrated that many plants release chemicals in response to larval feeding, and these volatiles have been shown to attract secondary insect predators (Turlings and Tumlinson, 1992; Tumlinson et al., 1993). In addition to the relationship of plant volatiles to insects, there also is interest in the volatiles emitted by plants as they relate to chemicals used in the flavor and perfume industry and other allied areas of research (Buttery, 1981).

Several indirect methods have been developed for elucidating potential volatile chemicals emitted from plants. Methods such as extraction with solvent or steam distillation used to identify "volatile" components from plants most often result in the identification of a large number of compounds that are not representative of the chemicals released by the plant (Tollsten and Bergstrom, 1988). Rearrangement and/or decomposition of many labile compounds often occur when many chemicals are subjected to heat or solvent. In addition to questions raised on the validity of compounds that are suggested to be "vola-

tilized," no quantitative data were obtained on the amount of the chemicals released.

Collection of chemicals from plants in a natural environment is difficult due to the fact that plants are sensitive to contact and movement such as wind [types of mechanically induced stress (MIS)]. Changes in growth rates and amounts of released volatile gases such as ethylene in response to these agitations has been documented (Biddington, 1986). Plant responses to stimuli become more pronounced with increased stress and this results in increased release of chemicals (Yu and Yang, 1980; Kimmerer and Kozlowski, 1982; Hyodo, 1991). Thus, it is not surprising that the cutting of flowers or sections of plants has been shown to alter the chemicals released from those plants (Heath et al., 1992). Because of this, it is important that the collection of plant volatiles is done in a manner that does not introduce stress on the plant.

Several systems have been designed to identify chemicals released from plants (Panasiuk, 1984; Loughrin et al., 1990b; Heath and Manukian, 1992). These systems were limited in the number of samples that could be collected during an experiment without having to go into the chamber to replace collection traps. Systems previously described typically were open at the bottom of the plant container and as such were subject to intrusion of unpurified air (Heath et al., 1992; Heath and Manukian, 1992). To prevent this from occurring, large volumes of pure air were required for positive purging of the test chamber, thereby allowing only a small percentage of the total released volatiles to be collected, which could potentially compromise accurate quantification of those chemical(s) released in trace amounts.

We describe here an automated system that can be used to collect chemical samples in any combination, sample volume, and time period in the plant's ambient surroundings with minimal stress placed on the plant. This system, using various electronic sensors, can simultaneously collect environmental data from the surroundings while the collection is occurring. To test the effectiveness of this system, we used ornamental red and yellow four o'clock flowering plants, *Mirabilis jalaba* (Nyctaginaceae), as a model to demonstrate the system's ability in collecting volatile chemicals.

METHODS AND MATERIALS

The entire system consists of three main parts, a guillotine collection assembly (GCA), an automated volatile collection system (AVCS), and an environmental data monitoring system (EDMS), each having several subcomponents. The GCAs are glass chambers into which the plants are inserted and from which volatiles are collected. The remaining two subsystems are used to control the overall experiment, make collections, and record all experimental parameters.

The AVCS consists of electronic hardware and pneumatics necessary for performing automated air sampling of volatiles from the GCAs. The EDMS consists of electronic sensors and hardware used to monitor environmental conditions affecting an experiment and store these data on a computer. Both the AVCS and EDMS can be used separately as independent modules or concurrently as one complete system. This is done through the MACDAS (Monitoring And Control, Data Acquisition System) for Windows control software developed specifically for this application at this laboratory (Manukian and Heath, 1993). The software contains separate modules for each system that can be multitasked in any combination on one computer using the Microsoft Windows 3.1 operating system.

Guillotine Collection Assembly (GCA). The GCA is shown in Figure 1. Air entering the system is purified by using five layers of 1.27-cm-thick carbon-infused polyester media containing 150% activated carbon (340 g fabric weight with 510 g carbon/sq yd, p/n ACF-NWPE-12-150P, Lewcott Corp., Millbury, Massachusetts) cut into 15.25-cm-diameter wafers. The use of this material to purify air in a manner that does not alter the ambient relative humidity has been described previously (Heath and Manukian, 1992). The five layers were compressed using an 11 metric ton Carver model 150-C hydraulic press (Menomonee Falls, Wisconsin) to a total load force of 1.1×10^5 newtons (24,000 lbf) applied to the filter media for 15 min. From the force of compression, the multiple layers are fused together, and the resulting disk is approximately 3 cm thick $\times$ 15.25 cm in diameter. Pressurized air (420 kPa), set at a constant flow at 5 liters/min by an adjustable flowmeter (Aalborg p/n P14/044-40ST, Monsey, New York), enters through a cap on top of the chamber and is diffused due to the back-pressure created by the compressed filters and a uniform air flow occurs in the test chamber.

The chamber used to house the plant is a 15.25-cm-OD $\times$ 40-cm-long $\times$ 0.4-cm-thick Pyrex glass tube with a 0.64-cm glass flange on the top end. The flanged end is used to seal the compressed carbon filters between the glass tube and air diffuser inlet cap using a 15.25-cm-ID phenolic coupler union (model S-6750-013, Southern Scientific Inc., Micanopy, Florida) (Figure 2). The glass test chamber with its attached filters is then placed on top of a multiport guillotine base using a 15.25-cm-ID $\times$ 7.62-cm-long piece of metal tube sleeve, which serves as a coupler to hold both sections together.

A multiport guillotine base (MGB) is used to close off the bottom of the test chamber around the stem of a plant. Two Teflon-coated blades, which come together to a tongue-and-groove joint with one half of a 2.54-cm hole cut into each blade, form a circular cutout around the stem of the plant resembling a guillotine (Figures 1 and 2). After the plant is inserted into the chamber, a piece of cotton is placed around the stem to prevent the plant stem from touching the MGB. Attached to the MGB above the closed blades, is a 15.25-cm-OD $\times$

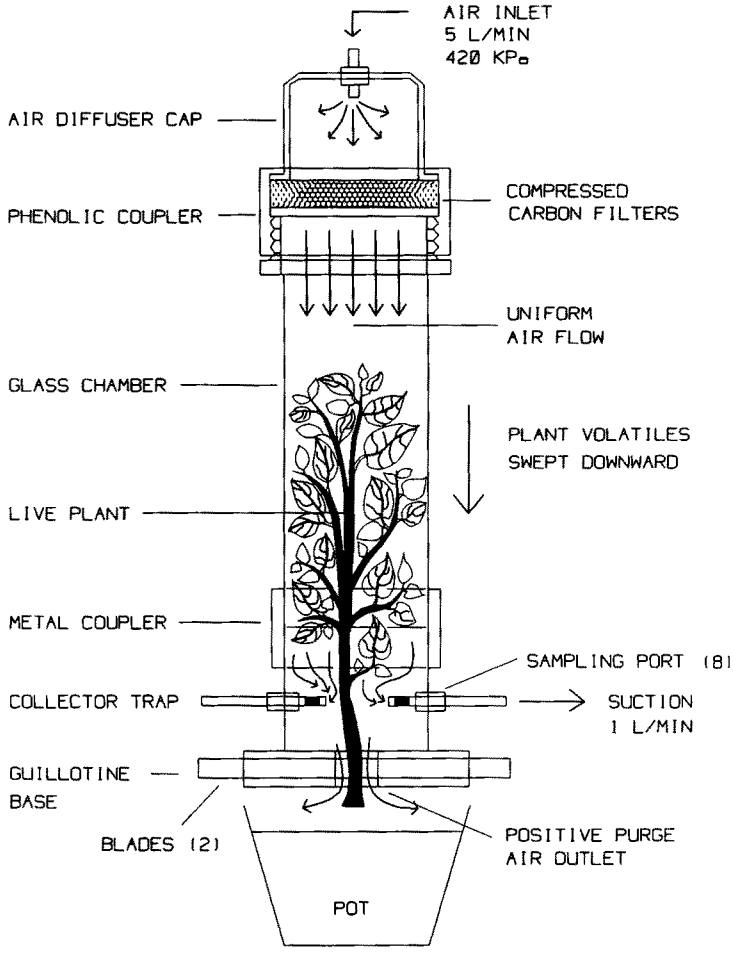

FIG. 1. 2-D side view/function diagram of the guillotine collection assembly (GCA) used for plant volatile sampling with the AVCS.

9-cm-long piece of 0.64-cm-thick clear acrylic Plexiglas tube with eight 0.64-cm Swagelok compression to M-NPT adapter fittings attached along the circumference of this tube at 45 degree angles from center, which act as filter ports for sampling. These bulkhead fittings serve as connectors for the volatile collector traps (VCT). The Plexiglas tube that contains the bulkhead connectors is lined with Teflon on the inside.

Monitoring And Control, Data Acquisition System (MACDAS). The auto-

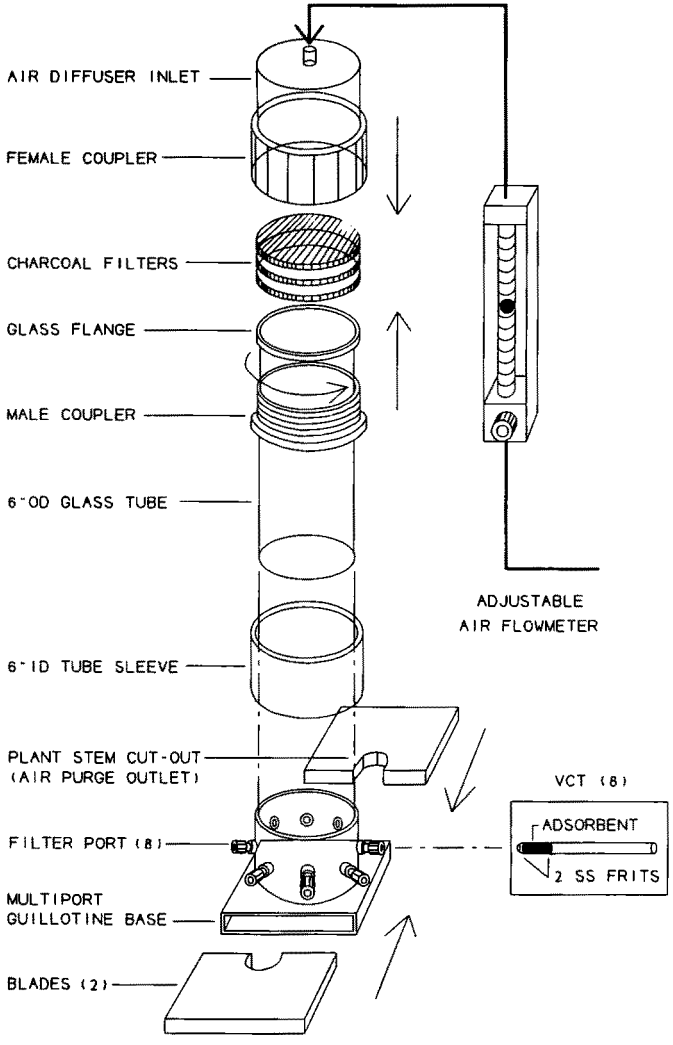

FIG. 2. 3-D exploded view of components of the guillotine collection assembly (GCA) used for plant volatile sampling with the AVCS.

mated volatile collection system (AVCS) and environmental data monitoring system (EDMS) used to set up, operate, and monitor all aspects of the system during an experiment has been described (Manukian and Heath, 1993). Briefly, a computer data system (CDS) consists of an Intel-based i80386DX-25Mhz CPU

computer capable of running Microsoft Windows 3.1 in the enhanced mode. Data acquisition is done using a ComputerBoards Inc., model CIO-DAS08 (Mansfield, Massachusetts) multifunction eight-channel analog and 32-channel digital I/O board. The digital section of the CIO-DAS08 is used for controlling the collection of air samples during an experiment, as well as controlling any additional external events by outputting control signals directed from the AVCS software module. This board contains 24 lines of bidirectional TTL digital I/O that are divided into three ports; A, B, and C, each containing eight channels, are configured as outputs used to control external solid state relays (SSRs), which act as electronic switches in turning on or off solenoid valves and equipment (Figure 3).

Two computer interface boards are used with the system to interface all external electrical hardware with the CIO-DAS08 data acquisition board (DAC) inside the computer. These boards are independent of each other and only share

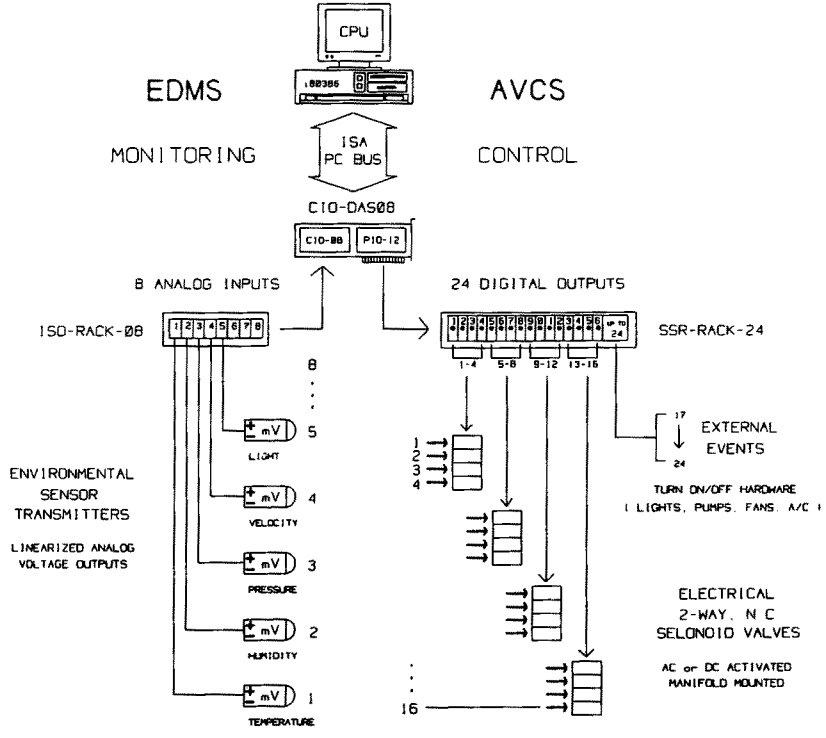

FIG. 3. AVCS and EDMS electrical components of the Monitoring And Control Data Acquisition System (MACDAS).

a common power source and electrical ground with the computer's main power supply. A ComputerBoards Inc., model SSR-RACK24 solid state relay rack equipped with 24 Grayhill Inc., type 70-OAC digital output AC modules (La Grange, Illinois) is used to turn the DAC boards TTL digital outputs directed from the AVCS module into control signals to turn on/off hardware and select samples for collection. A ComputerBoards Inc., model ISO-RACK08 universal analog isolation and interface board is used to isolate, amplify, filter, and condition all analog input sensory data from the ESTs into the EDMS module for display and storage.

To monitor environmental parameters associated with the experiment, we utilized an Omega Instruments model RH-411 digital thermohygrometer to record both temperature and relative humidity data (Stamford, Connecticut). For measuring light intensities during experiments, we used a three-decade digital light meter covering a range from 0 to 50,000 lux utilizing a selenium photovoltaic detector with a 300-nm bandwidth centered at 570 nm (Davis Instruments Inc., C/N# EH1191025, Baltimore, Maryland). Both of these instruments produced linearized mV analog outputs for input to the EDMS.

The selection of multiple samples for collection during an experiment is done through a sample switching manifold (SSM), which is controlled by the AVCS module in the MACDAS control software. This manifold consists of four separate banks of four, two-way, normally closed (NC), electrical (AC) solenoid valves, manifold-mounted with a common outlet (Versa Valves Inc., no. EZM-2180-4-0-243-120V60, Gulf Controls Corp., Tampa, Florida). Each bank is connected in parallel to a vacuum header, through a 0–1 liter/min adjustable flowmeter (Aalborg Instruments, p/n PO4/1-112-02C), which is in series with the outlet port of each bank of valves, making a total of 16 individual sampling lines that can be used in any combination. The parallel groups of four banks enable eight collections from up to two different chambers at the same time (Figure 4). The vacuum header is connected to a vacuum system, which consisted of a standard 115 liter (30-gallon) compressor tank with an oilless Teflon rotary vane vacuum pump (Gast model 1023-V126T-G272X, Gulf Controls Corp.).

Control of an experiment is achieved through the MACDAS for Windows software which was developed at this laboratory using the Microsoft Visual BASIC version 1.0 programming language along with the Microsoft Professional Toolkit for Visual BASIC and the DriverLINX/VB dynamic link library for accessing control functions of the DAC board through Windows (Scientific Software Tools, Inc., Malvern, Pennsylvania).

Volatile Collector Traps (VCT). The collector traps used were based on modification of traps previously described (Heath and Manukian, 1992). For this system the VCTs are made using a 12-cm-long × 0.4-cm-ID piece of glass tubing and contains 30 mg of 80/100 mesh Super-Q (Altech Assoc. Inc., cat.

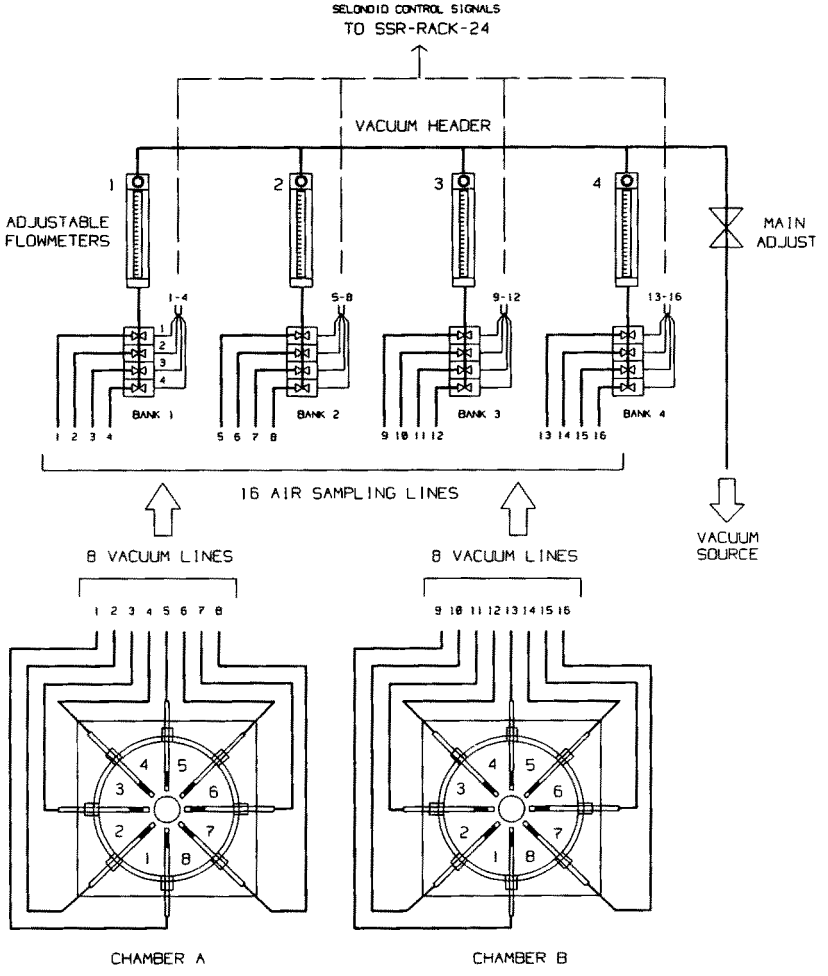

FIG. 4. Electrical and pneumatic schematic of the sample switching manifold (SSM) and vacuum line connections to multiport base of the GCA used for plant volatile sampling with the AVCS.

no. 2735, Deerfield, Illinois) as the adsorbent. Two 325-mesh stainless steel cloth frits are used to contain the adsorbent. The collector traps are connected directly to soft Tygon sampling lines from the SSM and are then inserted (adsorbent end first) through the filter ports in the guillotine base assembly (Figure 2, inset). Prior to use, the traps are cleaned by Soxhlet extraction using ultra-high purity methylene chloride for 24 hr. Volatiles collected on the traps are eluted

with 100 μl methylene chloride and then 20 ng of tridecan-1-ol acetate (*S*-C13 : Ac) is added as an internal standard for subsequent analyses and quantification in the laboratory.

Plants and Collection of Volatiles. Ornamental red and yellow four o'clock flowering plants *Mirabilis jalaba* (Nyctaginaceae), were grown individually in 2-liter pots containing a 50 : 50 mixture of common potting soil and vermiculite. Plants were grown in a greenhouse and collections of volatiles were made in November 1992. Two separate GSAs were used to collect volatiles from 6-week-old red and yellow four o'clock's, each containing one plant. At approximately 1130 hr, a GCA was placed over each plant and purified air at 5 liter/min was introduced into each chamber to purge the system. Volatile collections were made continuously using the AVCS starting at 1300 hr for 1 hr at a sampling rate of 1 liter/min, then continuing with samples taken at 2-hr intervals for a total period of 14 hr, ending at 0400 hr the following morning (total of eight samples). Each sample represented 20% of all chemicals present in the chamber during the period of each collection (5 liters/min total airflow entering at the top with 1 liter/min being sampled and the remaining 4 liters being vented through the bottom). The number of flowers completely opened or partially opened were counted at approximately 1700 and 1900 hr each day of collection. This process was continued for six consecutive days ($N = 6$) using both new red and yellow plants for each collection. In addition to the physical samples, environmental data (temperature, relative humidity, and light intensity) were collected continuously prior, during, and after each experiment using the EDMS.

Analysis of Volatiles. A Varian model 3400 GC and Finnigan ITD MS, equipped with a CTC-A200S 200 sample liquid automatic injector was used for analysis of plant volatiles. Typically, 10 μl of extract was injected into a septum programmable injector (SPI) for direct capillary cool on-column injection at 60°C. Zero grade helium (99.998%) was used as the carrier gas at a linear flow velocity of 20 cm/sec, and the temperature program was initially isothermal at 60°C for 5 min, then temperature programmed at 20°/min to 180°C. Capillary gas chromatography (CGC) was done using a combination of the two fused silica columns: first a 10-m × 0.25-mm-ID trimethylsilane deactivated fused silica retention gap column (Quadrex, New Haven, Connecticut) connected in series to the analytical capillary column, then a 30-m × 0.25-mm-ID Supelcowax 10 (bonded Carbowax) with a 0.25-μm-film thickness, also purchased from Quadrex, using GlasSeal connectors (Supelco Inc., Bellefonte, Pennsylvania). This system permitted the on-column injection of samples without concentration in 5–100 μl of solvent (Grob, 1982; Murphy, 1989). The detector end of the analytical column was coupled to the source of a Finnigan MAT Ion Trap Detector mass spectrometer (ITD-MS) or a Varian flame ionization detector (FID). GC-MS spectra were obtained using electron impact (EI) and analysis of the spectra data was done using the Finnigan-MAT Trapmaster software. The

GC/FID chromatographic peak quantitation was processed using the Perkin-Elmer/Nelson TurboChrom3 software.

RESULTS AND DISCUSSION

Several major modifications in the design of systems previously described (Heath et al., 1992; Heath and Manukian, 1992) for the collection of volatiles from plants were incorporated into the system presented in this paper. During initial attempts to increase the diameter of the carbon filter disks from 9.5 cm to 15.25 cm for this larger system, we observed that the multiple layers of the 1.27-cm-thick carbon filter media would separate, acting as individual filters and thus the purity of air entering the chamber was unacceptable. By fusing the multiple layers of this charcoal media together using extreme pressures, the resulting single fused filter disk produced an excellent means of purifying air in addition to circumventing the problems associated when trying to handle the multiple, thinner disks.

The open-ended system previously used required large volumes of air to ensure that the air velocity through the test chamber was sufficient to prevent intrusion of unpurified ambient air into the chamber. The development of the GCA provides an easy method of incorporating multiple collector traps and simultaneously resolving the problem of ambient air intrusion into the purified air chamber by restricting the open end of this chamber to a minimum. The degree of air purification within the chamber is shown by comparing typical gas chromatograms of the volatiles released during the 1800- to 2000-hr (peak release) period and absence of volatiles observed during the 0200- to 0400-hr (nominal background) period (Figure 5). As seen in the gas chromatograms, impurities from the air inside the chamber are minimal during the nominal background periods and the amount of material released from a plant, which is in the low nanogram (< 10 ng) range, is easily detected.

In the course of our evaluation of the system, we used four o'clocks as the plant model. The major compound released was identified as 3,7-dimethyl 1,3,7-octatriene (ocimene) based on mass spectroscopy (GC-MS). Confirmation of the identity of ocimene was based on comparison of mass spectra and retention time of the collected natural material with synthetic standard material (Ocimene # 15-1353/Lot # SK061188, International Flavors & Fragrances Inc., New York, New York, CAS# 502-99-8). Several other compounds also were identified in some of the volatile collections from four o'clocks. These included: (Z)-3-hexenyl acetate, 7-methyl 3-methylene 1,6-octadiene (myrcene), benzaldehyde, and indole. None of these compounds were found consistently and their occurrence was not related to a particular time period.

The average percent of ocimene released from four o'clocks during the

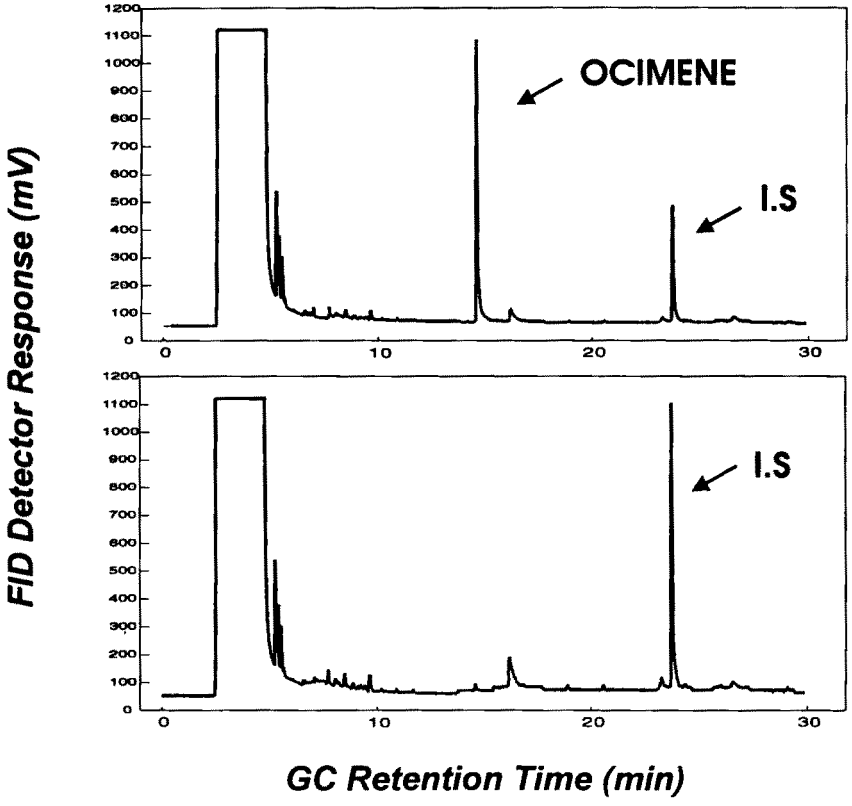

FIG. 5. Upper trace is a typical gas chromatogram obtained of volatiles released from four o'clocks during 1800–2000 hr. Lower trace is a typical gas chromatogram obtained when no volatiles are released from four o'clocks during 0200–0400 hr. The internal standard (IS) is 20 ng of *S*-C13 : Ac.

various time periods is shown in Figure 6. Onset of chemical release occurred during the 1400- to 1600-hr time period, and the greatest amount of ocimene was released between 1800 and 2000 hr. Release of ocimene then decreased during the 2000- to 2400-hr time period. Very little ocimene was released after 2400 hr. Determination of the amount of ocimene released per flower was calculated for the 1800- to 2000-hr time period. Based on the number of fully open and partially opened flowers, we were able to determine that yellow four o'clocks released 80.9 (±7.3 SD) ng ocimene/flower/hr and that the red four o'clocks released 51.9 (±7.0 SD) ng ocimene/flower/hr. The amount of ocimene released by yellow flowered four o'clock's was significantly more than

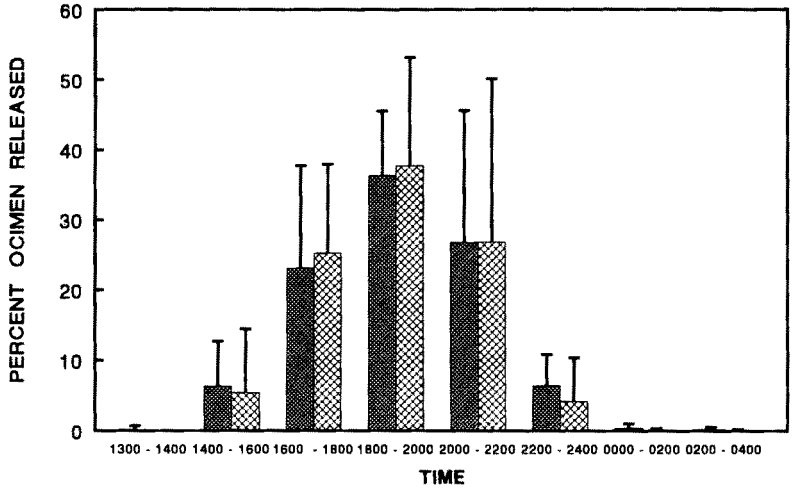

FIG. 6. Average percent ($N = 6$) of ocimene released by four o'clocks at different time periods. Cross-hatch bars are percent ocimene released from red flowers and slanted striped bars are percent ocimene released from yellow flowers. Error bars indicate SD.

that released by yellow flowered four o'clocks was significantly more than that released from red flowered four o'clocks ($t = 6.42$, $df = 10$, $P = 0.0001$). Although the timing of release for ocimene was similar for both the red and yellow flowering plants, the yellow flowers released more material than the red flowers.

Examination of environmental data recorded during the six experiments indicated a high degree of variability in light intensity, temperature, and humidity. This largely was due to the dynamic weather changes experienced daily in Florida during this time of year. A representative data set of continuous measurements of temperature, humidity, and light intensity obtained during an experiment is shown in Figure 7. Considerable fluctuation in light intensity occurs between 1200 and 1700 hr. These fluctuations reflect the sensitivity and the responsiveness of the light sensor and EDMS to varying cloud coverage that occurred during a relatively sunny day. Light intensity decreased rapidly after 1700 hr with darkness (<2 lux) occurring at approximately 1840 hr. Temperature was fairly stable, with an overall drop in temperature throughout the test. Relative humidity, being inversely related to temperature, showed an increase as both light intensity and temperature dropped after 1430 hr.

The system described here affords the simultaneous identification of volatile chemicals released from plants and an automated record of environmental conditions that occurred during the investigation. Automation allows for continuous

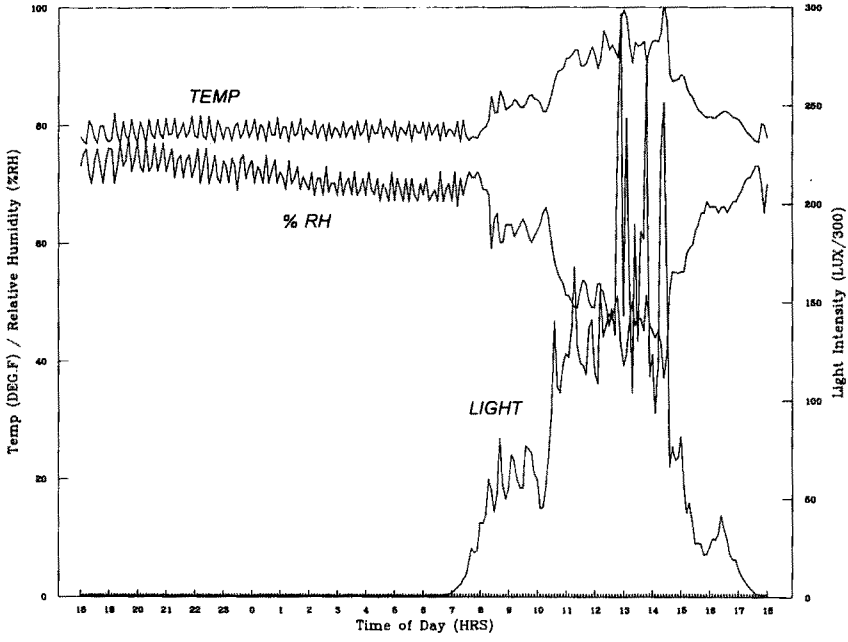

FIG. 7. Plot of environmental conditions recorded from the EDMS during a 24-hr period covering a typical volatile collection. Temperature, relative humidity, and light intensity are measured at a frequency of 20 KHz and are averaged and recorded every 6 min.

around-the-clock experimentation without requiring the presence of a human operator. This, in turn, should increase research productivity by decreasing the time taken to obtain experimental data and minimize the chance of contaminating the test chamber atmosphere or artificially inducing stress upon the plant through contact. Use of the described system offers a myriad of research opportunities including the determination of volatiles released from different varieties of crops, volatiles released from plants due to environmental stress and larval feeding damage, and determination of the effect of plants on pheromone released from pest insects.

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REFERENCES

ALTENBURGER, R., and MATILE, P. 1988. Circadian rhythmicity emission of fragrance in flowers of *Hoya carnosa* R. Br. *Planta* 174:248–252.

ALTENBURGER, R., and MATILE, P. 1990. Further observations on rhythmic emission of fragrance in flowers. *Plant* 180:194–197.

BIDDINGTON, N.L. 1986. The effects of mechanically-induced stress in plants—a review. *Plant Growth Regul.* 00:101–123.

BUTTERY, R.G. 1981. Vegetable and Fruit Flavors, pp. 175–216, *in* R. Teranishi, R.A. Flath, and H. Sugisawa (eds.). Flavor Research, Recent Advances. Marcel Dekker, New York.

GROB, J.J. 1982. Band broadening in space and the retention gap in capillary gas chromatography. *J. Chromatogr.* 237:15–23.

HEATH, R.R., and MANUKIAN, A. 1992. Development and evaluation of systems to collect volatile semiochemicals from insects and plants using a charcoal-infused medium for air purification. *J. Chem. Ecol.* 18(7):1209–1226.

HEATH, R.R., LANDOLT, P.J., DUEBEN, B., and LENCZEWSKI, B. 1992. Identification of floral compounds of night-blooming jessamine attractive to cabbage looper moths. *Environ. Entomol.* 21(4):854–859.

HYODO, H. 1991. Stress/wound ethylene, pp. 43–63, *in* A.K. Mattoo and J.C. Suttle (eds.). The Plant Hormone Ethylene. CRC Press, Boca Raton, Florida.

KIMMERER, T.W., and KOZLOWSKI, T.T. 1982. Ethylene, ethane, acetaldehyde, and ethanol production by plants under stress. *Plant Physiol.* 69:840–847.

LOUGHRIN, J.H., HAMILTON-KEMP, T.R., ANDERSEN, R.A., and HILDEBRAND, D.F. 1990a. Headspace compounds from flowers of *Nicotiana tabacum* and related species. *J. Agric. Food Chem.* 38(2):455–460.

LOUGHRIN, J.H., HAMILTON-KEMP, T.R., ANDERSEN, R.A., and HILDEBRAND, D.F. 1990b. Volatiles from flowers of *Nicotania sylvestris*, *N. otophora* and *Malus x domesetica*: Headspace components and day/night changes in their relative concentrations. *Phytochemistry* 29(8):2473–2477.

LOUGHRIN, J.H., HAMILTON-KEMP, T.R., ANDERSEN, R.A., and HILDEBRAND, D.F. 1991. Circadian rhythm of volatile emission from flowers of *Nicotiana sylvestris* and *N. suaveolens*. *Physiol. Plant.* 83:492–496.

MANUKIAN, A., and HEATH, R.R. 1993. Development of an automated system to simultaneously collect volatiles and monitor environmental conditions for use in life sciences research. *Sci. Comput. Autom.* 9(11):27–40.

MATILE, P., and ALTENBURGER, R. 1988. Rhythms of fragrance emission in flowers. *Planta* 174:242–247.

MURPHY, R.E. 1989. The fractionation gap: An optimized coupling of fused silica columns in open tubular gas chromatography. Master's thesis. University of Florida, Gainesville.

PANASIUK, O. 1984. Response of Colorado potato beetles, *Leptinotarsa decemlineata* (Say), to volatile compounds of tansy, *Tanacetum vulgare*. *J. Chem. Ecol.* 10(9):1325–1333.

TOLLSTEN, L., and BERGSTROM, G. 1988. Headspace volatiles of whole plants and macerated plant parts of *Brassica* and *Sinapis*. *Phytochemistry* 27:4013–4018.

TUMLINSON, J.H., LEWIS, W.J., and VET, L.E.M. 1993. How parasitic wasps find their hosts. *Sci. Am.* 268:100–106.

TURLINGS, T.C.J., and TUMLINSON, J.H. 1992. Systemic release of chemical signals by herbivore-injured corn. *Proc. Natl. Acad. Sci. U.S.A.* 89:8399–8402.

WYATT, R. 1983. Pollinator-plant interactions and the evolution of breeding systems, pp. 51–95, *in* L. Real (ed.). Pollination Biology. Academic Press, New York.

YU, Y.-B., and YANG, S.A. 1980. Biosynthesis of wound ethylene. *Plant Physiol.* 66:281–285.